

Exhibit G

FACS Analysis of Transfected cells w/ Following Vectors

pCDNA 7

FT7, 1, 2a, 2b, 3

1, 2b, 3

1, 3

cDNA 3

cDNA 10

cDNA 14

2 plates / Vector - Divide

FACS 7mls

yuko 5mls

EAT 3mls (75ul)

ET assay 5mls (100ul)

Antibodies

IgM H - black 1:100 /

IgM hrf green 1:1000

IgM SLrf 1:200

IgG ha red 1:500

IgG SLa blue 1:500

2nd Antibody

IgM 2.5mls

12.5 / 2.5mls

IgG 1.5

60 / 1.5mls

Results are

H - all neg

hrf - all neg

SLrf, pCD (-), 1, 2a, 2b, 3(+), 1, 2b, 3(+), 1, 3 (-), cDNA 3(-), 10 (+), 14 (+)

ha all neg

SLa all neg

Sperm 12 sperm KG, FT4

6451

6080

2420

6199

6374

6087

6306

6086

6203

6085

5721

5671

Run sig gel of above samples

Also sperm

5728

6084

7213

5731

5732

6082

5662

5727

6201

5725

5672

6200

Spinning gel of samples
Sequence

FT4 6079

6202

6307

6373

CDNA10 715

946

CDNA14

T7

8850

8807

8874

Protein assay of FACS Samples, Also CAT assay

pcDNA

FT7, 1,2a2b,3

FT7, 1,2b,3

FT7, 1,3

CDNA3

CDNA10

CDNA14

BSA Blank Protein

0 1.00 116 108

1 0.208 225 214

2 0.369 383 376

4 0.691 673 682

8 1.215 1.230 1.222

16

Sample

pcDNA 0.292 0.294 0.293

1,2a2b,3 0.337 0.330 0.333

1,2b,3 0.298 0.343 0.320

1,3 0.369 0.372 0.370

CDNA3 0.363 0.379 0.371

CDNA10 0.306 0.298 0.302

CDNA14 0.225 0.253 0.23

FACS Results

Only stain w/ shx

1,2a2b,3 23.6%

1,2b,3 24.6%

CDNA10 14.9%

CDNA14 8.0%

Micro BCA Protein Assay

Reagent mic	MC	MB	MA
Per assay tube (ml)	0.01	0.24	0.25
Cocktail for Tubes			

Incubate 1 h at 60°C and cool to room temp.

Since the color development has no end point, all tubes must be heated and cooled at the same time.

1 mg/ml BSA (l)	Water (l)	Reagent (l)	Abs. 562	
0.0	500.0	500.0	Blank	Slope = 0.0734 Y intercept = 0.0656 X intercept = -0.8940 R = 0.9985
1.0	499.0	500.0	0.108	
2.0	498.0	500.0	0.214	
4.0	496.0	500.0	0.376	
8.0	492.0	500.0	0.682	
16.0	484.0	500.0	1.222	

Spinning of FT4 samples

Sample	l in assay	Water (l)	Reagent (l)	Abs. 562	mg protein/ml
pcDNA1	5.00	495.00	500	0.293	0.62
FT7 1,2a2b,3	5.00	495.00	500	0.333	0.73
FT7 1,2b,3	5.00	495.00	500	0.320	0.69
FT7 1,3	5.00	495.00	500	0.370	0.83
CDNA 3	5.00	495.00	500	0.371	0.83
CDNA 10	5.00	495.00	500	0.302	0.64
CDNA 14	5.00	495.00	500	0.239	0.47

CAT Assay (FT7) samples

2.5 μ l of cell extract

Control

pcDNA

1,2a2b,3

1,2b,3

1,3

cDNA 3

cDNA 10

cDNA 14

Cocktail 15

3/4 Chlor 300

Tris, 2M pH 8.0 75

But Co A 75

H₂O 30050/ μ l tube

CAT

Vector	Counts/5ul		Incorporated Counts (.95)		Total Counts	Total Counts Incorporated	
pcDNA1	11,349	11,829	9,189	8,063	238,189	244,643	9,673
FT7 1,2a2b,3	11,161	11,441	27,211	21,919	250,431	250,739	28,643
FT7 1,2b,3	11,772	11,826	37,541	40,684	272,981	277,204	39,517
FT7 1,3	11,215	11,690	23,076	28,708	247,378	262,506	24,291
cDNA 3	11,834	11,206	33,885	39,096	270,565	263,216	35,668
cDNA 10	12,017	11,312	30,066	33,165	270,406	259,405	31,848
cDNA 14	11,079	11,570	44,133	40,529	285,713	271,929	46,456
Control		10,354		424		207,504	446
Protein Conc. (ug/2.5ul)		Total Counts Inc- Bkg	% INC/hr		% INC/hr/ug		Mean CAT Activity
pcDNA1	1.55	9,249	8,063	3.92	3.30	2.53	2.33
FT7 1,2a2b,3	1.83	28,219	22,649	11.27	9.03	6.17	4.95
FT7 1,2b,3	1.72	39,093	42,401	14.32	15.30	8.33	8.89
FT7 1,3	2.07	23,867	29,793	9.65	11.35	4.66	5.48
cDNA 3	2.07	35,244	40,730	13.03	15.47	6.29	7.48
cDNA 10	1.60	31,224	34,487	11.55	13.29	7.22	8.31
cDNA 14	1.17	46,032	42,238	17.32	15.53	14.81	13.28

Double Data of FT7 FACS, CAT assay / give to Juko
 Work on FT4 samples

7 days sequencing on Troublesome FT4 samples

6451 6200
 6378 6079
 6306 6307
 6203 1897
 5721 1898
 1899

Sequencing gel of samples (FT4) Formamide gel
 Probe Brownie within But w/ GAP probe
 To check condition of RMT

The 7 days technique didn't resolve all of the compressions
 Try a Terminal transferase technique

Run standard Syntexase rxn, after extension reaction
 Heat tubes (A, C, G, T) for 1.5 mins 100°C
 Hold on ice 10 min, Prepare TdT/dNTP cocktail
 Add to tubes, 37°C 30 min
 Add 5ul Stop